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ADP019696

TITLE: Mammalian Cell Interactions with Nanophase Materials

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TITLE: Materials Research Society Symposium Proceedings. Volume 845, 2005. Nanoscale Materials Science in Biology and Medicine, Held in Boston, MA on 28 November-2 December 2004

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Mammalian Cell Interactions with Nanophase Materials

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ABSTRACT

Adhesion of differentiated mammalian cells from various hard and soft tissues, including adult mesenchymal stem cells is different on nanophase than on microphase/conventional ceramics (such as alumina, titania and hydroxylapatite) as well as on composites of these ceramics with either poly(L-lactic) acid or poly(methyl) methacrylate. Most importantly, nanophase materials promote selective interactions, for example, of osteoblasts but not of fibroblasts. The type, amount and conformation of adsorbed proteins (such as fibronectin, collagen and vitronectin) are key aspects of the underlying mechanism(s) of subsequent cell interactions with nanophase materials. These cellular/molecular results provide evidence that nanophase biomaterials have the potential for improving the efficacy of implants and for promoting neotissue formation pertinent to tissue engineering, regenerative medicine and other clinical applications.

INTRODUCTION

Current research on implantable biomaterials is motivated by the need for biomaterials with properties similar to those of the physiological tissues they are intended to replace. In addition there is the requirement that for the clinical success of implants, it is not enough to minimize undesirable tissue-biomaterial interactions. It is also necessary to promote specific functions of surrounding cells and tissues. In this respect, nanostructured materials (with grain sizes less than 100 nm in at least one dimension) are extremely promising. Supporting evidence has been provided by numerous, recent, in vitro studies, that utilized mammalian cells from various hard and soft tissues.

CELL ADHESION

Among cell functions, adhesion is of primary and crucial importance for anchorage-dependent cells because, when they do not adhere, those cells do not survive. Although cell proliferation can be expected to follow adhesion, proliferation (and other subsequent cell functions) need to be determined in separate and independent studies (specifically designed for that purpose) before definitive conclusions can be drawn.

Understandably then, adhesion of mammalian cells to nanophase materials was the first function examined by researchers in this field. The first, statistically-significant

evidence of enhanced adhesion of rat calvaria osteoblasts on flat, nanophase (versus microphase/conventional) ceramics (alumina, titania and hydroxylapatite) as a function of decreasing ceramic grain size was provided only a few years ago [1-2]. Most importantly, the enhanced adhesion was specific to osteoblasts, but neither to fibroblasts nor to endothelial cells [3]. Furthermore, the select preference and specific enhanced adhesion of osteoblasts was maintained only on composites of poly (L-lactic) acid (PLA) and nanophase (but not on polymer/conventional) ceramics (alumina, titania and hydroxylapatite) composites [4]. Specifically, osteoblast adhesion on the 50:50 (w:w)% PLA/nanophase alumina composite was similar to that obtained on 100% nanophase ceramic (the maximum observed in the study) In addition, these material formulations exhibited improved mechanical (specifically, bending modulus) properties [4].

It should be noted that nanophase ceramics and their composites promoted and supported cell adhesion (and subsequent cell functions such as proliferation [2]) that were previously observed only on material surfaces modified through laborious and lengthy processes that involved immobilization of specially-designed, bioactive, synthetic adhesive peptides [5-7]. In contrast, preparation of the nanophase ceramics and their composites was easy, straight-forward and, in terms of time, short (on the order of a few hours).

In contrast, adult mesenchymal stem cell adhesion was maximal on microphase (1,500 nm) ceramics (alumina, titania and hydroxylapatite), decreased with material grain size, and was the lowest observed on the nanophase (50 nm) grain size ceramics tested [8]. In order to reconcile any discrepancies in the trends of mammalian cell adhesion on nanophase material substrates, further investigations of the underlying molecular mechanisms are needed.

MECHANISMS OF CELL ADHESION: THE ROLE OF PROTEINS

It is well established and widely accepted that proteins (specifically, the type, amount, and conformation) adsorbed on surfaces of substrates modulate and control subsequent adhesion and other functions of cells. Evidence that this type of mediation is involved in the adhesion of mammalian cells on nanophase ceramics was provided by data that, in the absence of serum proteins (a standard supplement in cell culture media), adhesion of osteoblasts was very low and similar on both nanophase and conventional/microphase ceramic substrates tested [9]. In fact, compared to results obtained on conventional/microphase alumina, the concentration of total adsorbed proteins from those present in media supplemented with 10% fetal bovine serum was the highest on nanophase alumina [3]. Most importantly, the adsorption trends observed on conventional/microphase alumina and nanophase alumina were different for the proteins tested, specifically, similar on the two substrates for albumin, enhanced on conventional/microphase alumina for laminin, but enhanced on nanophase alumina for denatured collagen, fibronectin and vitronectin [3]. Since denatured collagen, fibronectin and vitronectin are adhesive proteins known to mediate bone cell interactions with

substrates, these results provided an explanation for the observed, enhanced osteoblast adhesion on nanophase alumina on which each one of these three proteins was per-adsorbed [3]. Further research in this respect revealed other details of the underlying mechanisms, specifically, that conformation of adsorbed vitronectin on nanophase alumina promoted exposure and/or activation of Arginine-Glycine-Aspartic acid (RGD) epitopes [10], which are known ligands recognized by specific receptors on osteoblast membranes [11-12].

Recent research also provided evidence that adsorbed proteins dictated subsequent cell adhesion on nanophase ceramics. Specifically, when fibronectin, but not vitronectin (a known, specific mediator of osteoblast adhesion), is preferentially adsorbed on nanophase (50 nm grain size) alumina, osteoblast adhesion is decreased compared to that observed on conventional/microphase (either 200 or 1,500 nm grain size) flat, alumina substrates [8].

THE FUTURE OF NANOPHASE MATERIALS IN BIOMEDICAL APPLICATIONS

Undoubtedly, understanding the mechanisms underlying protein and cell interactions with synthetic substrates (including nanophase ones) will lead to improved implant biomaterials and biomedical devices. Since proteins mediate adhesion of mammalian cells and, therefore, are the crucial aspect of the mechanism(s) underlying mammalian cell interactions on nanophase materials, further theoretical and experimental studies are needed to elucidate the mechanism(s) of protein interactions with nanophase material surfaces. It is possible that, once pertinent properties of nanophase materials are identified, definitively studied, and consistently reproduced, these material formulations will provide manufacturable substrates with known trends of protein adsorption and predictable, subsequent, select and specific mammalian cell interactions. Such material formulations could have major impact in tissue engineering, regenerative medicine and other clinical applications.

ACKNOWLEDGMENTS

Results of the theses-related work of my former graduate students (in alphabetical order) Dr. Kay C Dee, Ms. Meredith E. Hasenbein, Ms. Anastasia J. McManus, Dr. David A. Puleo, and Dr. Thomas J. Webster as well as of current doctoral candidate Mr. Aaron J. Dulgar-Tulloch were presented in this paper. These research endeavors were also collaborations with the following colleagues (in alphabetical order): Dr. Thomas T. Andersen of Albany Medical College as well as Dr. Robert H. Doremus, Dr. Linda S. Schadler and Dr. Richard W. Siegel of the Department of Materials Science and Engineering and the Rensselaer Nanotechnology Center of Rensselaer Polytechnic Institute. Dr. Harry Kimelberg and Ms. Carol Charniga of Albany Medical College

provided the rat calvariae (source of the osteoblasts used in the studies). Nanophase Technologies, Inc., provided some of the alumina and titania powders. Part of the research was funded by a grant by Philip Morris USA. I wish to take this opportunity to express my thanks and appreciation to all these individuals and organizations for their respective contributions to the research endeavors described in this paper.

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